An *in silico* model of endotoxic shock mediators

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14. ABSTRACT
Biologically-based *in silico* models of pathogen-host interactions are being designed in our lab to predict the time-course of pathogenic infection in humans. Macrophages respond to lipopolysaccharides (LPS), including the release of potent lipid autacoids, causing a cascade of events leading to endotoxic shock. However, animals have been shown to vary in response and susceptibility to *E. coli* endotoxin: guinea pig > hamster > mouse. To establish a sound basis for interspecies extrapolation, a pathogenesis model is being extended to encompass endotoxic shock. Exposing experimental animals to aerosols of LPS elicits bronchoconstriction, activation of alveolar macrophages, and recruitment of inflammatory cells into airways. These effects have been attributed to a potent lipid autacoid, platelet-activating factor (PAF). Species differences in the biomodulatory effects and mechanisms of PAF are similar to those seen with endotoxin. In guinea pigs, PAF (2 ug/kg IV) causes bronchoconstriction and hypotension in seconds and lethality within 25 minutes. In rats, however, 3 ug/kg of PAF had a negligible impact on heart rate. Therefore, a dynamic model for PAF was developed to link a pathogen's kinetics and host response. The current model focuses on kinetics and receptor binding of PAF and its antagonist ginkgolide B (GB). The kinetic models include plasma, red blood cell, lung, heart, and rapidly and slowly perfused tissues, with IV and inhalation exposure routes, and pathways for binding and elimination of PAF. Kinetic parameters were from the literature. The model was used to simulate experimental exposures to PAF and GB, revealing potential explanations for species differences in sensitivity to PAF. Internal dose metrics were generated and correlated with observed signs of infection and lethality in an attempt to identify the most appropriate metrics for predicting adverse effects. This model of pathogen kinetics and these dose metrics help to elucidate mechanisms of host response dynamics and improve cross-species extrapolation of response data.

15. SUBJECT TERMS
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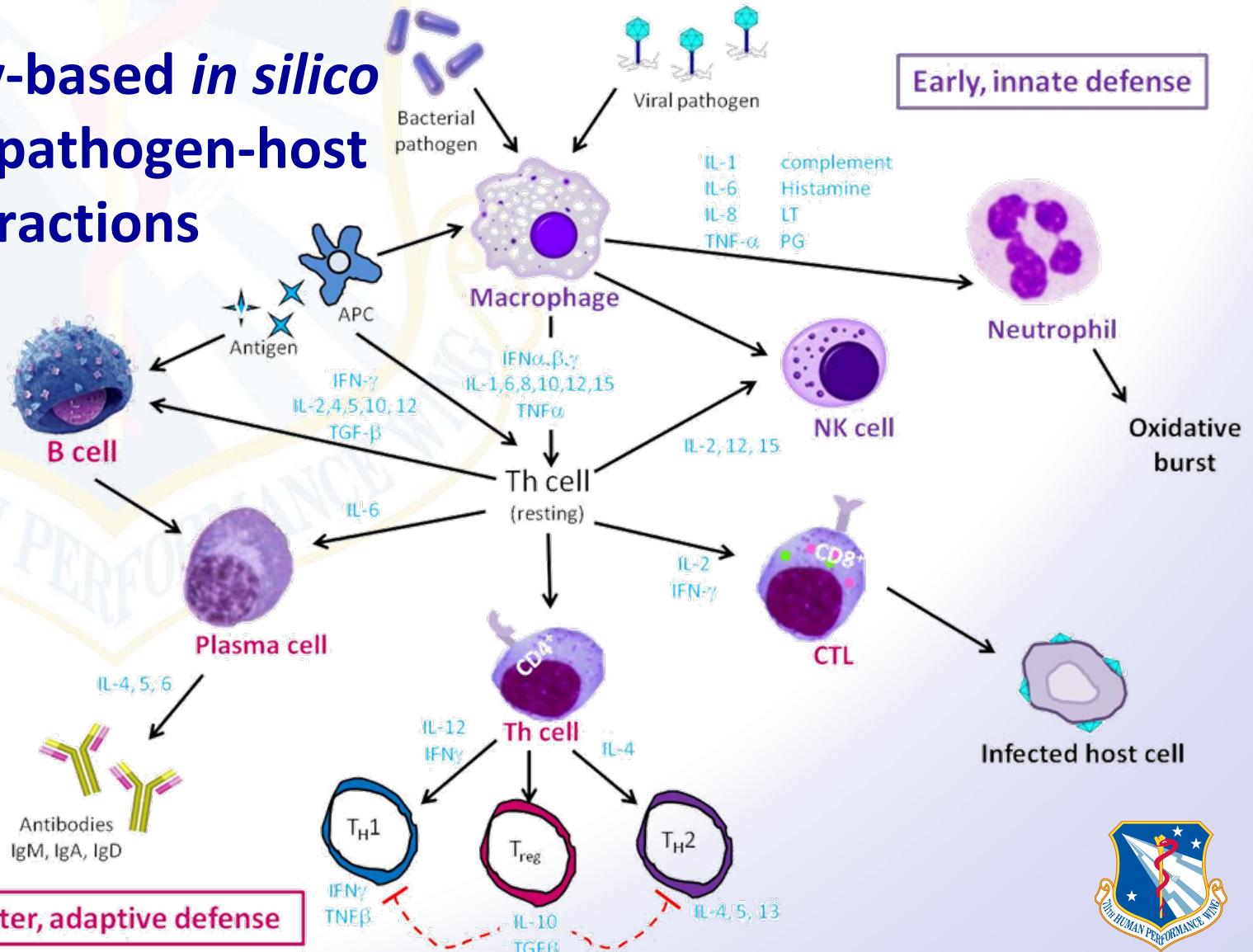
Our ongoing efforts

Biologically-based *in silico* models of pathogen-host interactions



Our ongoing efforts

Immune system response model

- Predict time-course of pathogenic infection in humans
- Quantify systemic response to pathogen exposure
- *F. tularensis* as our case study

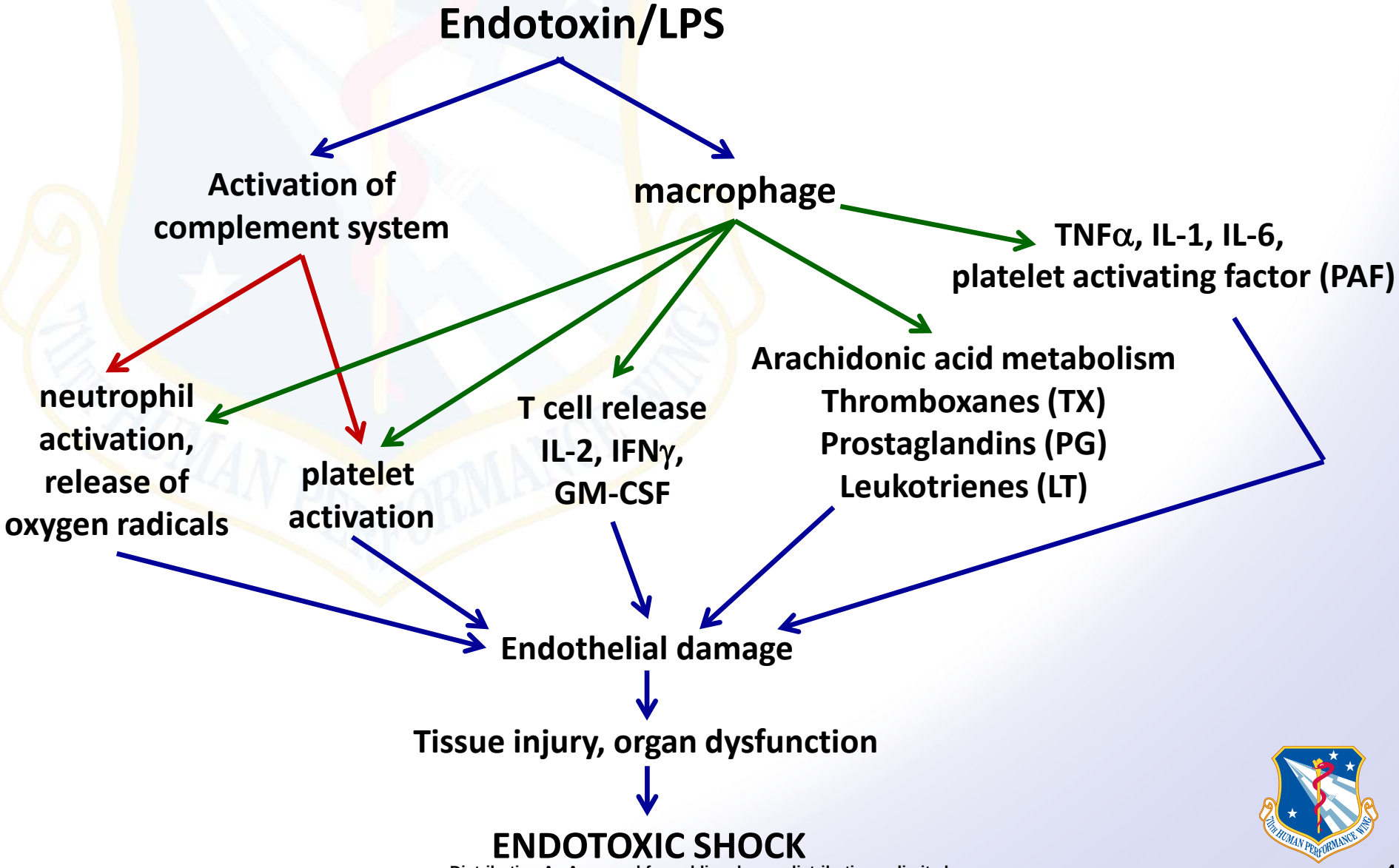
Need a 'bridge' to eventual health outcome

- Mediators of shock

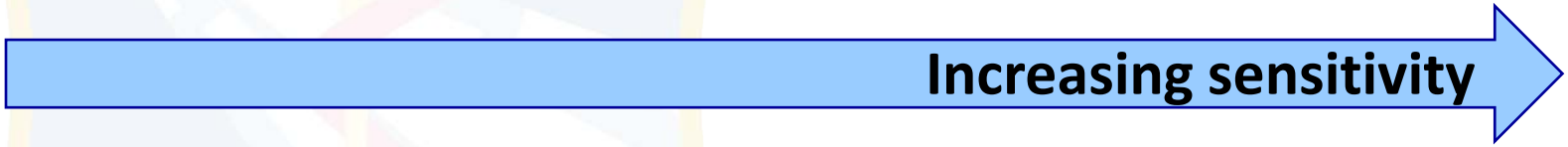
Endotoxin/lipopolysaccharide (LPS)

Principal component of gram-negative bacteria cell wall

Mediators of endotoxic shock



Species differences in sensitivity to *E. coli* endotoxin



mouse
rat

macaques

human

guinea pig
rabbit

Reason(s) for species-dependent sensitivity to endotoxin?

Hypothesis – Due to differences in mediator kinetics/dynamics?

Vasoactive lipid mediators in endotoxic shock

Eicosanoids can be detected in circulation at high enough concentrations to be responsible for events in endotoxic shock

- High PG levels in circulation of animals subjected to endotoxin
- Increased plasma TXB_2 in humans suffering from severe septic shock
- Endotoxemia and sepsis: Blood PAF levels are elevated

Vasoactive lipid mediators in endotoxic shock

Synthesis inhibitors or receptor antagonists of lipid mediators are capable of modifying the course of endotoxic shock

- Lipoxygenase (LOX) inhibitors protect mice and rats from lethal endotoxemia
- TXA_2 synthetase inhibitors are effective in rat endotoxic shock
- TXA_2 receptor antagonists block development of pulmonary hypertension in endotoxemia
- PAF antagonists (Ginkgolide B, GB) protect rats, mice, pigs, and humans from injurious effects of LPS

Aims

To elucidate wide range of sensitivity to LPS between species

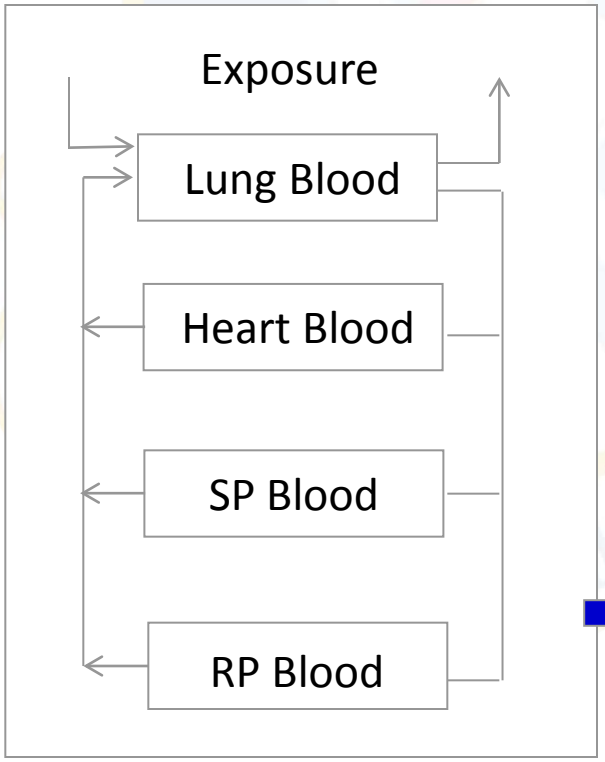
- Looked into differences in kinetics and dynamic responses to downstream mediators (PAF)
- Literature suggests species differences in PAF response in guinea pig, human, and rat (in decreasing order of sensitivity)

Aims

Develop a biologically-based *in silico* model of PAF to give insights into dynamic evolution of endotoxic shock

- Elucidate link between kinetics and biological response
- Simulate kinetic data reported in literature
- Extrapolate animal response data to humans

Iterative approach to modeling

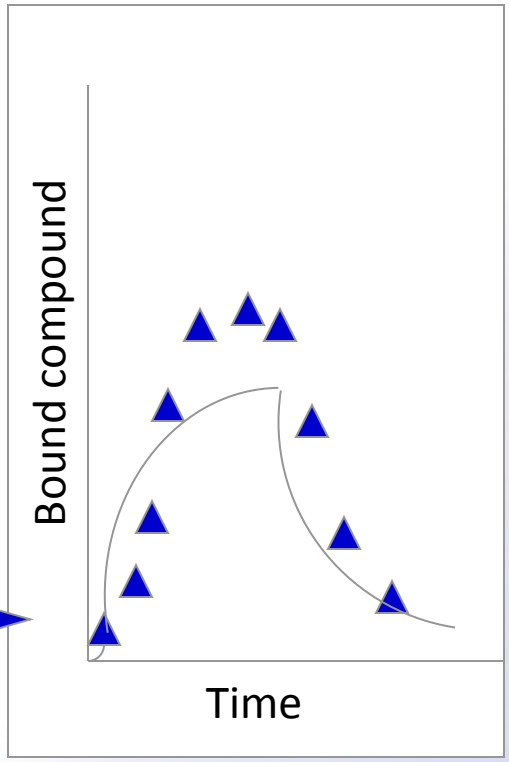


Physiological Parameters
Tissue volume
Blood flow rates

PAF kinetics
Partition
Binding
Density
Hydrolysis

+

Model Equations



Define Realistic Model

Collect Data For Each Species

Make Predictions

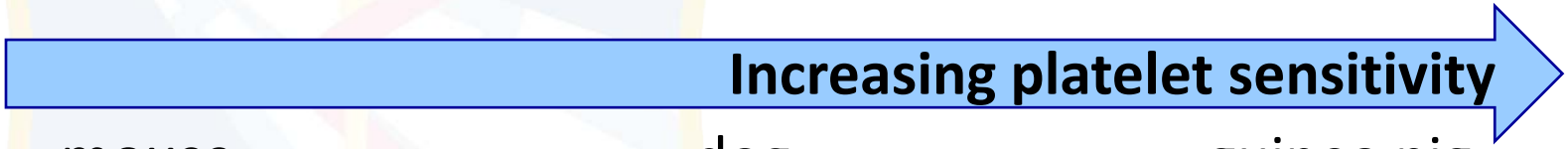
Refine Model

Platelet-activating factor (PAF)

- Autacoid binds to specific PAF receptor sites
  - Affinity and density is dependent on cell, tissue, and species
- Much data in literature focus on characterization of PAF binding to receptors on platelets
- While PAF effects are universal, platelet sensitivity towards PAF receptor varies among species
- **Species-dependent difference at receptor level of platelets**

rat mouse	Rhesus, cebus apella primate	human baboon canine	guinea pig rabbit

Platelet sensitivity is correlated to in vivo responses to PAF



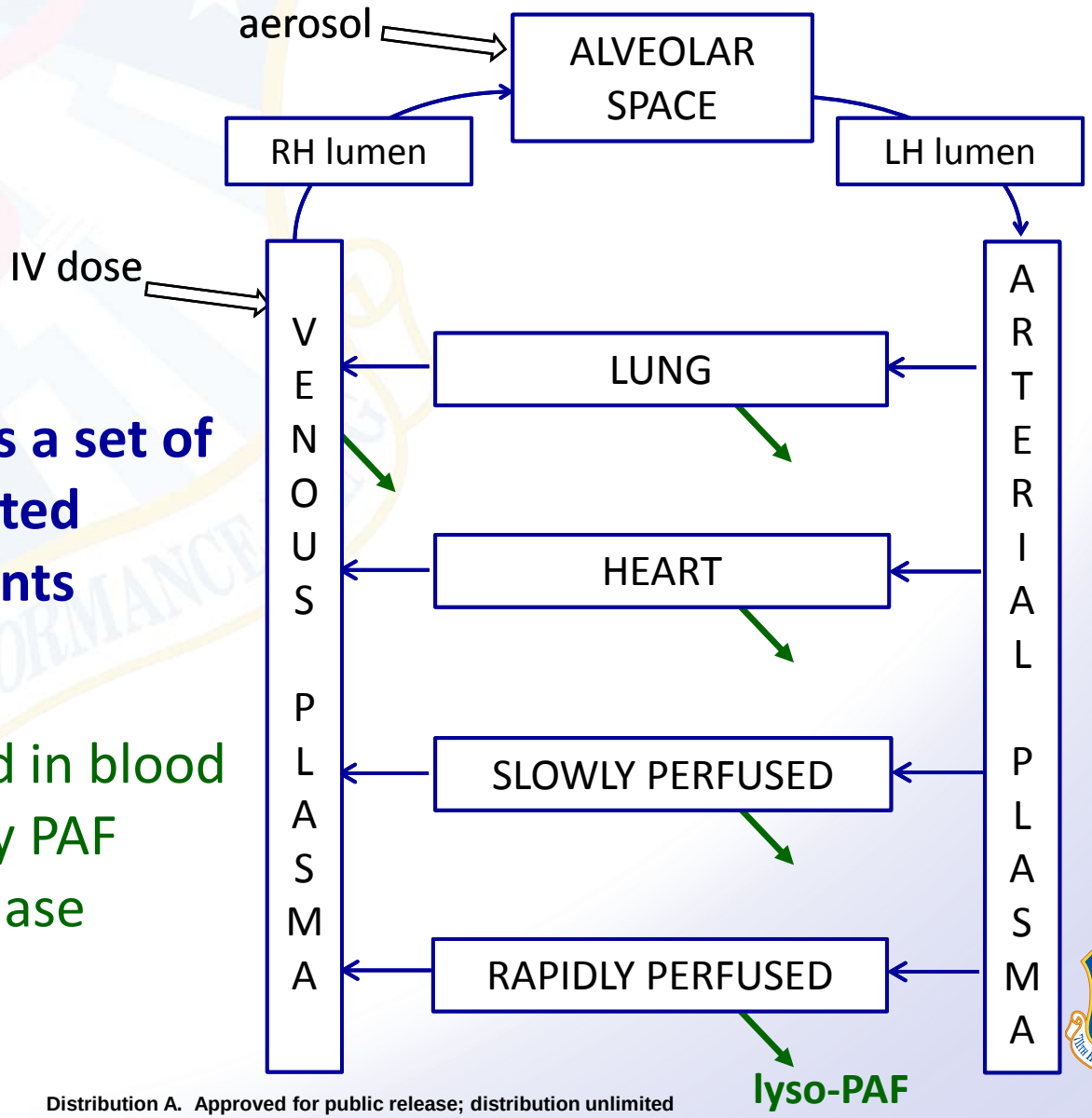
mouse	dog	guinea pig
rat	primate	rabbit
reduced/absent bronchoconstrictive responses	human	bronchoconstriction
hemoconcentration at higher doses		hemoconcentration

Platelet sensitivity is correlated to *in vivo* responses to PAF

We can extrapolate *in vitro* cell line data to whole tissues, and from there to whole animals with PBPK models

- C16-PAF shows slightly higher, but statistically insignificant difference in potency between human lung and platelets
  - **Similarity between PAF receptors in human platelets and lung tissue**
- ***In vitro* platelet binding data were used in our models at tissue level when tissue-specific data were unavailable**

INITIAL PBPK model schematic for endogenous PAF

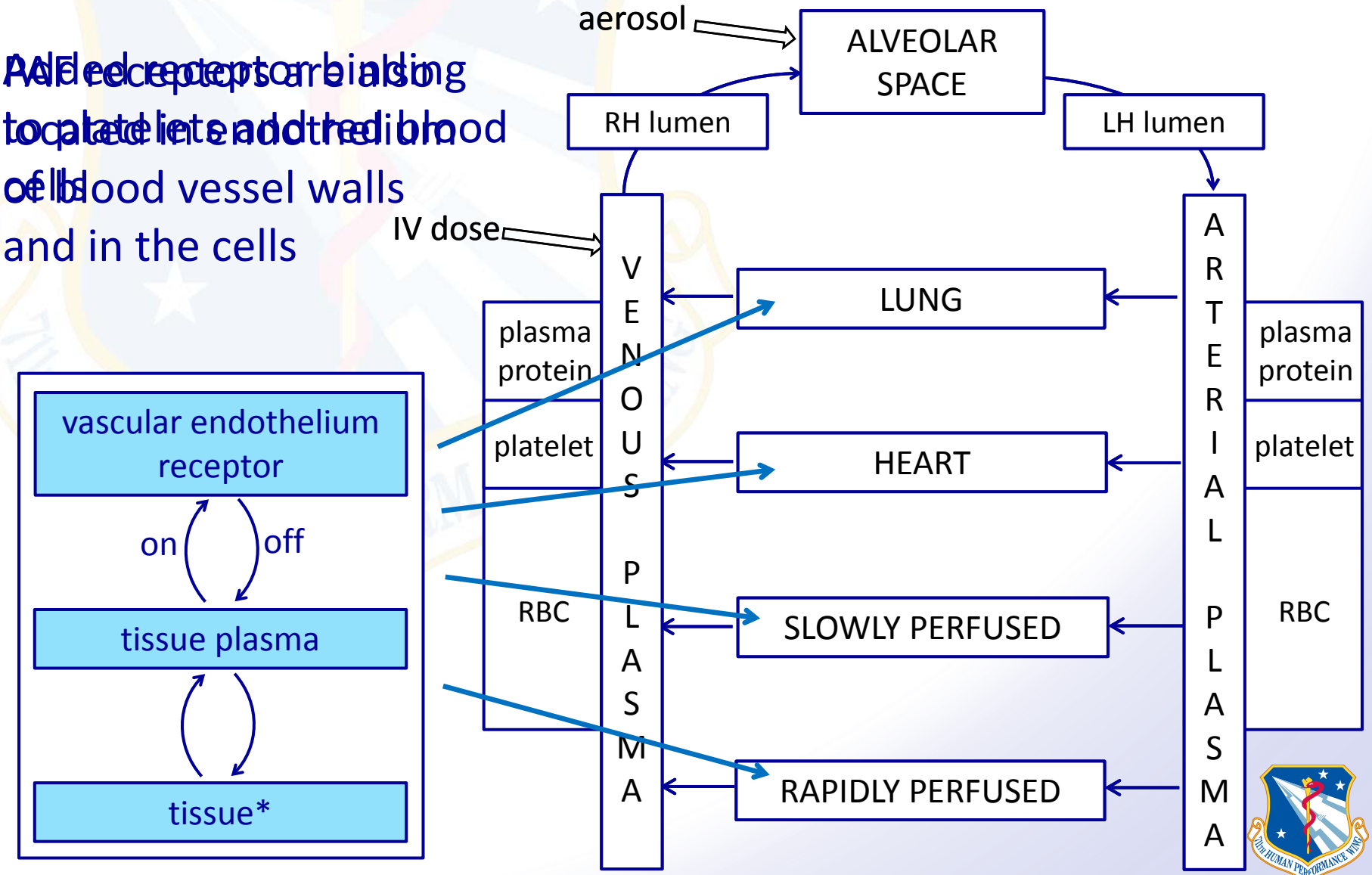


Describes body as a set of interconnected compartments

PAF is metabolized in blood and tissues by PAF acetylhydrolase

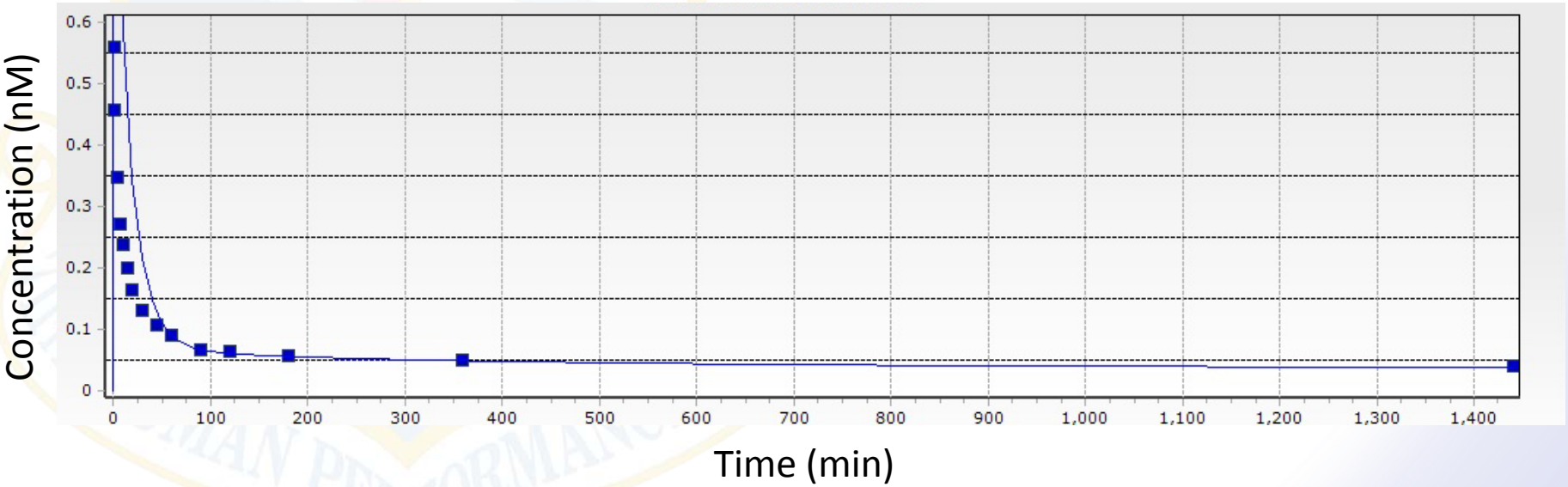
IMPROVED PBPK model schematic for endogenous PAF

PAF receptors are also located in endothelium of blood vessel walls and in the cells



The resulting integrated model is used to simulate pharmacokinetics of PAF after intravenous exposure…

Pharmacokinetics of PAF (1.25 μg/kg IV)

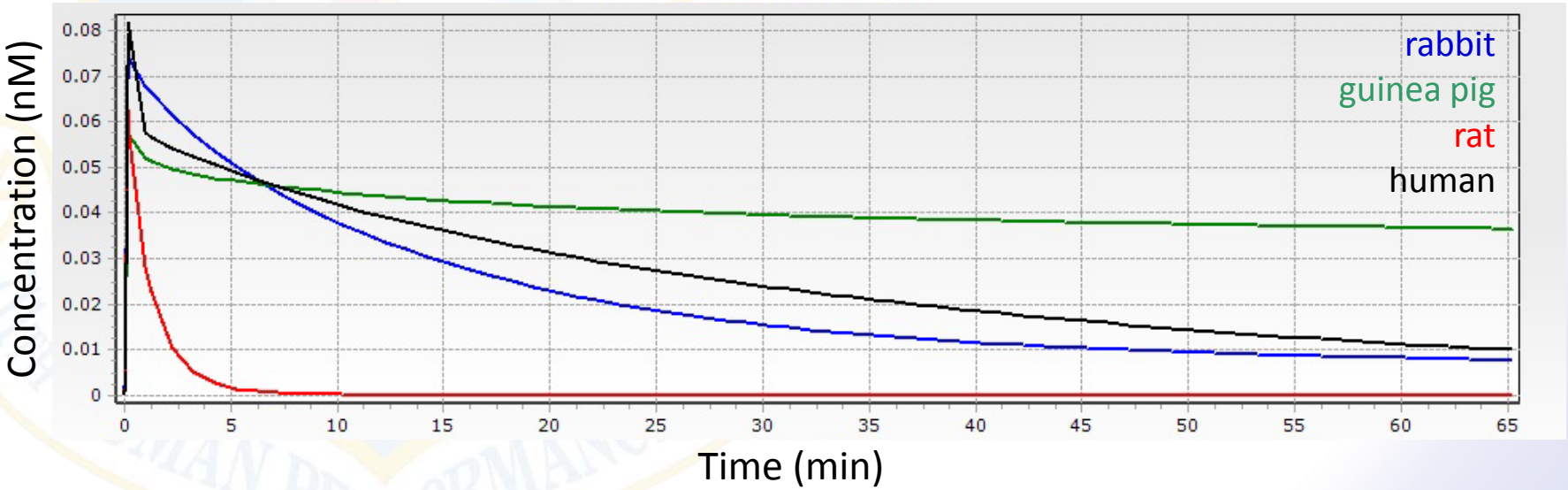


Simulation of [^{3}H]PAF concentration in venous plasma in rabbit following a 1.25 μg/kg IV exposure compared to data

- This simulation compared with kinetic data show that the model is capable of accurately simulating the experimental data

Experimental data: Lartigue-Mattei *et al.*, 1994

Pharmacokinetics of PAF (1.25 μg/kg IV)

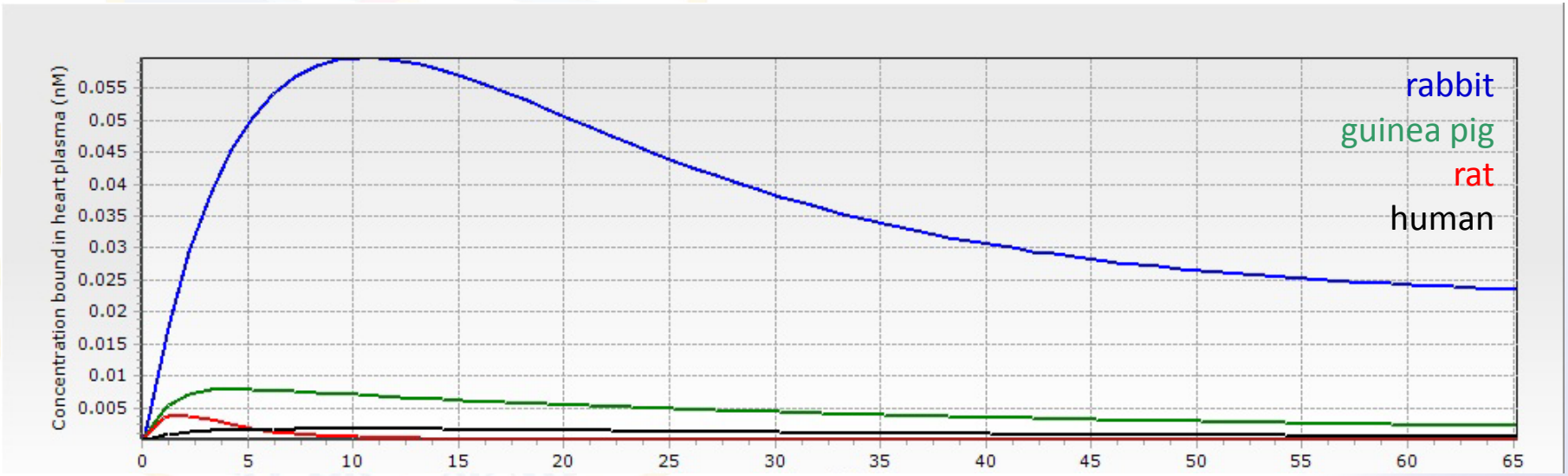


Data show a link between differential sensitivity to LPS and PAF among species and PAF kinetic parameters

- Higher plasma PAF in guinea pig corresponds to its increased susceptibility to LPS

Experimental data: Lartigue-Mattei *et al.*, 1994

Pharmacokinetics of PAF (1.25 µg/kg IV)



Simulations demonstrate that bound PAF in heart plasma is higher in rabbit than rat

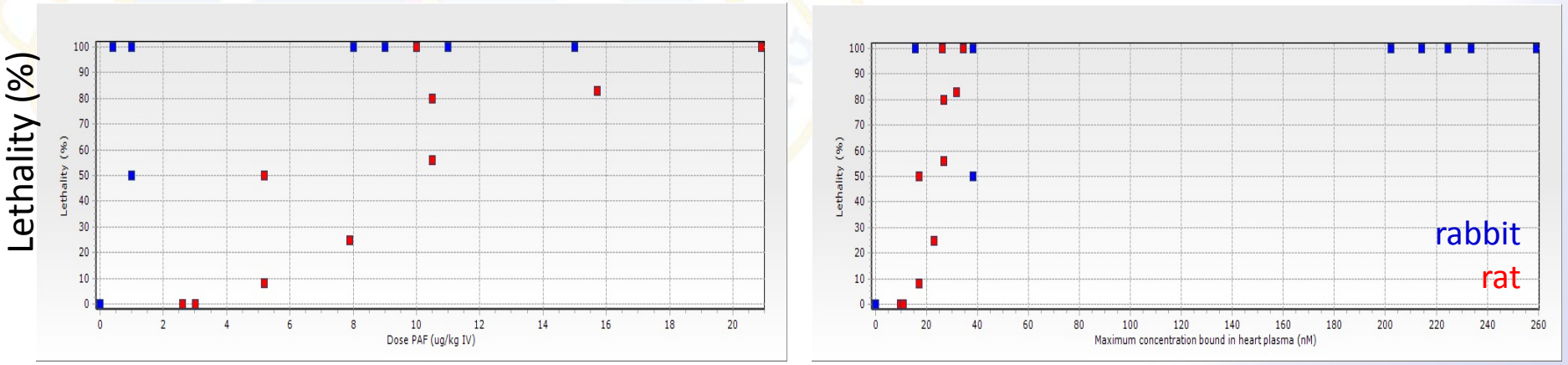
- Possibly explains why it is more toxic in rabbit than rat

Experimental data: Lartigue-Mattei *et al.*, 1994

Model predictions of external LD_{50}

- Various model outputs were examined for correlation with observed signs of infection and lethality in an attempt to identify the most appropriate dose metrics for predicting adverse effects

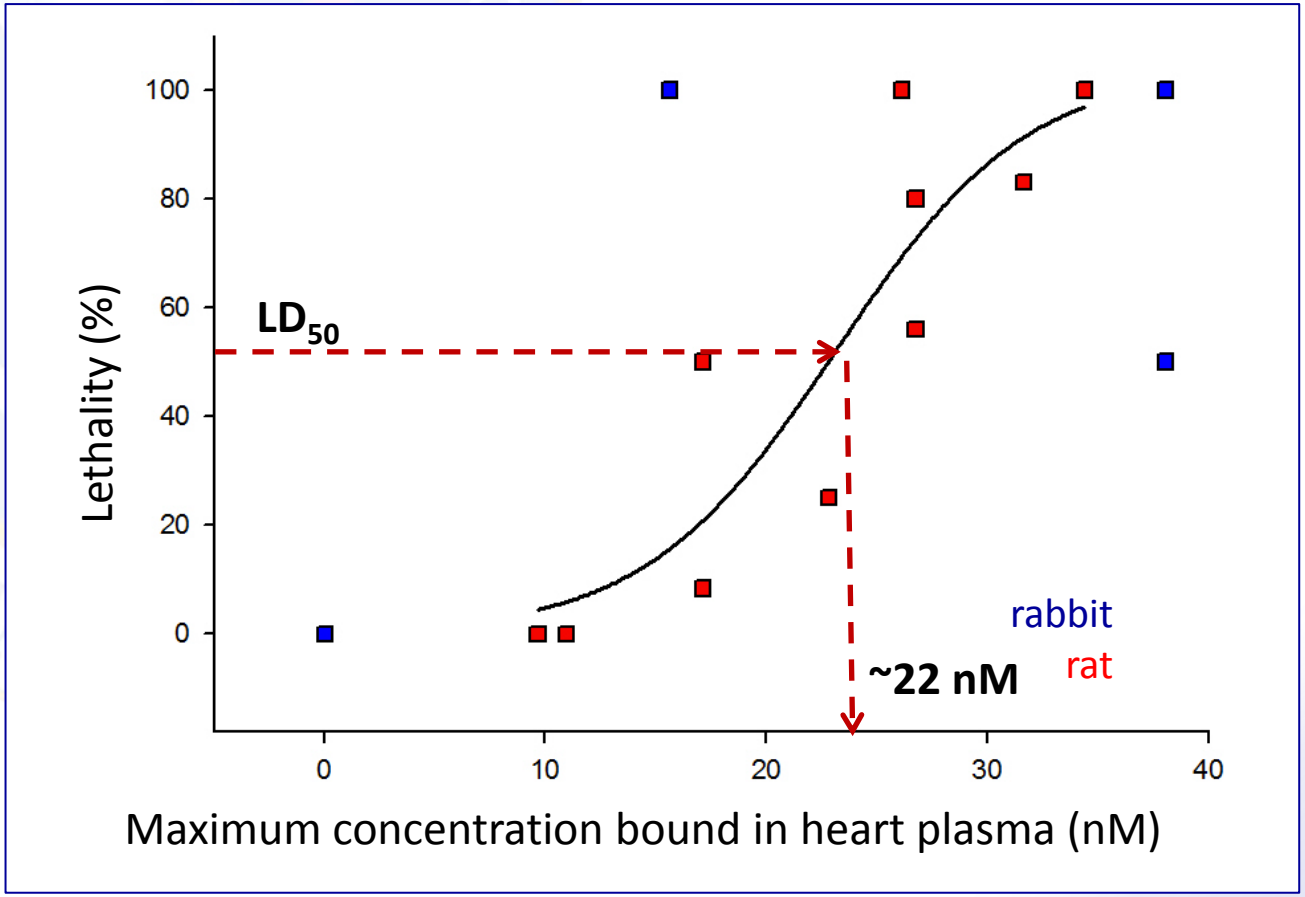
Lethality dose response curve



Peak concentration of bound compound in heart plasma

Experimental data: Lefer *et al*., 1984, Tanaka *et al*., 1983

Lethality *vs.* internal dose metric



Regression of lethality on the internal dose metric results in an internal LD_{50} of ~22 nM peak bound in heart plasma

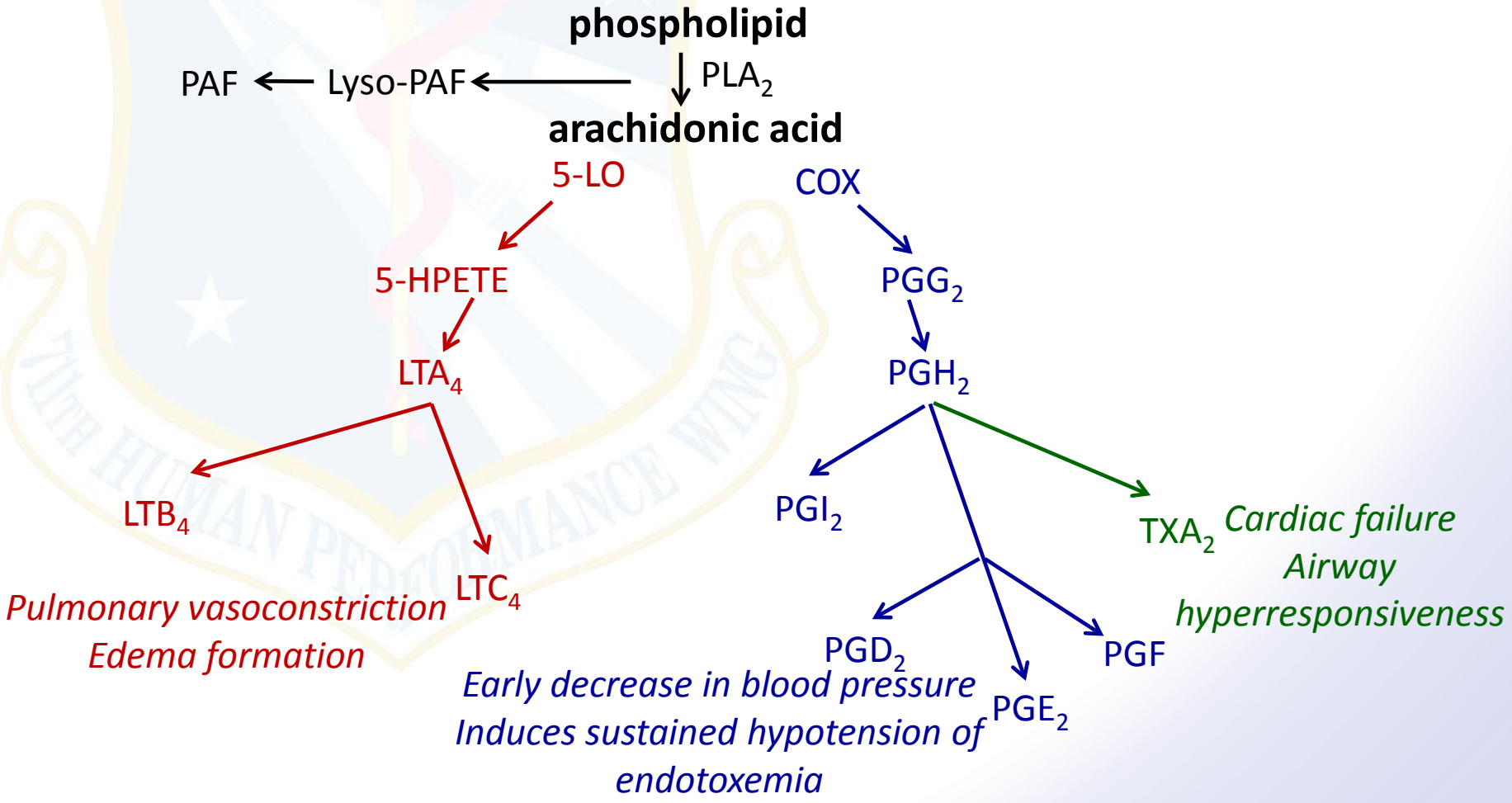
Human lethality prediction

- Running the human model shows that the human external dose required to achieve this peak is 900 μg/kg IV
  - Humans should exhibit intermediate clearance/binding, consistent with intermediate toxicity of PAF in humans

	Rabbit	Human	Rat
External LD50 (μg/kg IV)	0.57	900	7.5

- Wrong dose metric?
- Dynamic differences downstream from receptor binding?
- Future work – *in vitro* experiments to verify value

Arachidonic acid (AA) cascade Species differences



Interspecies differences in eicosanoid involvement highlight the need to consider the AA cascade in interpreting toxicity data

Future work

- Additional comparisons with kinetic and toxicity data in literature
- Confirmation of human parameter values used in human lethality prediction
- Addition of other exposure routes in order to simulate realistic human exposure scenarios (inhalation)

Adapt model to antagonists

PAF antagonists protect against injurious effects of LPS

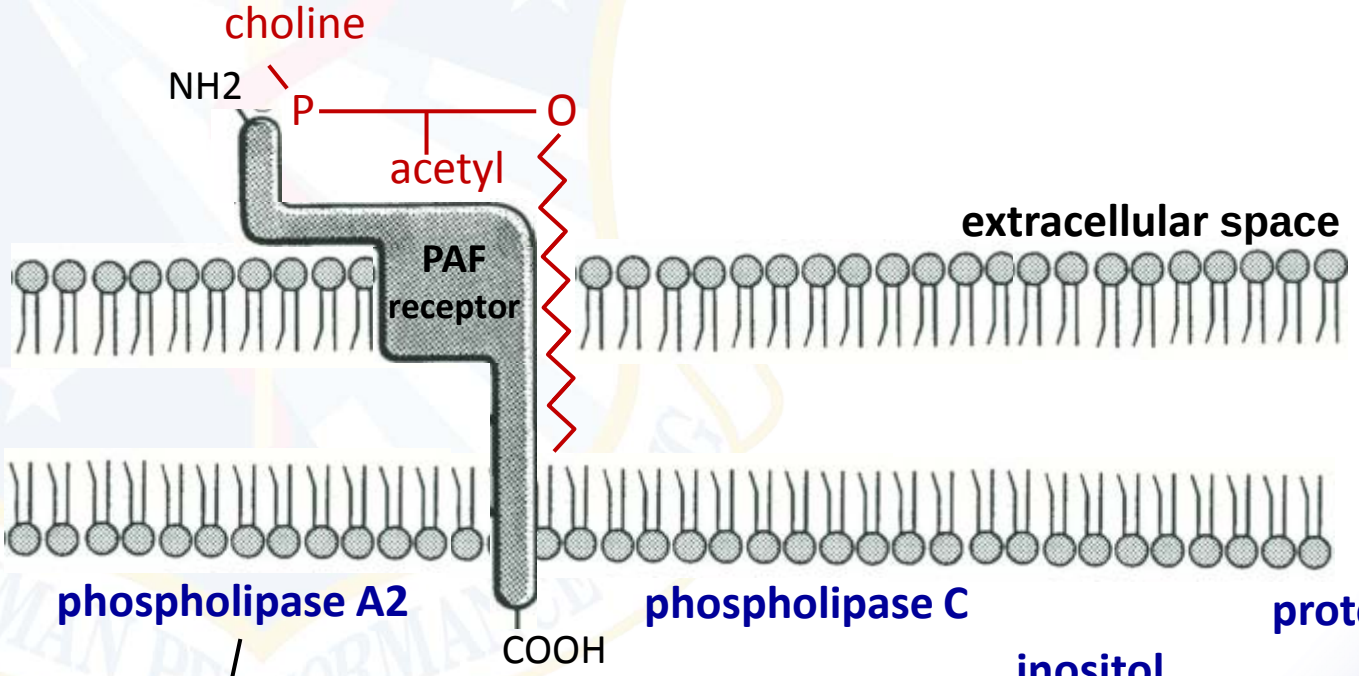
- GB inhibits PAF-induced platelet aggregation and attenuates airway vascular permeability, hypotension, and lethality, and also AA accumulation
- Preincubation of platelets with GB diminished PAF binding in a specific and saturable manner

Model structures for PAF analogs and antagonists are similar

- Different physicochemical and biochemical parameter values

Modeling the kinetics of these antagonists and interactions/competition with PAF for the receptor would evaluate **therapeutic efficacy**

Model refinement



Mechanisms involved in bioactions of PAF are complex probably because receptor activates multiple signaling pathways

Summary

Model of PAF response interactions has been developed

- Describes distribution to tissues, hydrolysis, and binding to receptors in tissue vasculature following IV exposure
- Capable of accurate simulation of kinetic profiles in animals
- Peak PAF bound in heart is indicated as a dose metric for extrapolation of lethality across species

Model development is an iterative process

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